ACS Infectious Diseases

Viewpoint

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Teixobactin and Its Analogues: A New Hope in Antibiotic Discovery

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ABSTRACT: Increasing bacterial resistance against current antibiotics and lack of new molecules to combat bacterial resistance are key challenges to global health. There is, therefore, a continuing need to develop new antibiotics. Teixobactin, a cyclic undecapeptide, displays excellent antibacterial activities against a range of pathogenic bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis*. Interestingly, it operates by multiple modes of actions and is bactericidal toward *S. aureus* without detectable resistance. This unique combination of wide Gram-positive activity coupled with its inability to elicit resistance make teixobactin a very attractive molecule for antimicrobial therapeutic development. This Viewpoint discusses teixobactin, its analogues, and the challenges and opportunities associated with their future development.

By 2050, antimicrobial resistance (AMR) is predicted to be responsible for more deaths than cancer.[1] There is an extremely high demand for new antibiotics with novel modes of action to combat the rampant spread of resistance encumbering classic therapeutic agents. One such recently discovered molecule is teixobactin,[2] which has shown excellent activity against a range of clinically relevant, Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium tuberculosis*, and *Enterococcus spp.* (vancomycin-resistant enterococci, VRE).

Teixobactin was isolated from a new species of previously uncultured Gram-negative bacteria provisionally named *Eleftheria terrae* via use of iChip culturing technology.[2] Teixobactin is a cyclic undecapeptide comprised of four D-amino acids, namely, *N*-Me-D-Phe$_1$, D-Gln$_4$, D-*allo*-Ile$_5$, and D-Thr$_8$, and an unusual L-*allo*-enduracididine amino acid (Figure 1A). The putative biosynthetic cluster for this depsipeptide natural product consists of two large (~697 kDa) nonribosomal petide synthetases (NRPSs) Txo1 and Txo2. The latter enzyme, Txo2, contains two consecutive thioesterase domains on the terminal module, a rare feature within NRPSs. The producing bacteria export teixobactin across their outer membrane where it exerts antimicrobial action against potentially competitive organisms. There have been no other protective resistance mechanisms reported in *E. terrae* against teixobactin, implying that resistance through horizontal gene transfer from the producing organism is improbable.[2]

The bactericidal activity of teixobactin against *S. aureus* is superior to vancomycin, and it retains excellent bactericidal activity against vancomycin-intermediate resistant *S. aureus* (VISA). Teixobactin is not effective against Gram negative bacteria, most likely due to the outer membrane barrier, as evidenced by the fact that teixobactin showed good activity against the outer membrane deficient strain *Escherichia coli asmB1*.[2] Most interestingly, no resistant mutants have emerged from *S. aureus* and grown under subminimum inhibitory concentrations (MIC). This absence of any detectable resistant mutants after serial passaging makes teixobactin a particularly attractive molecule for drug development and is suggestive of a nonprotein drug target. Treatment of *S. aureus* with teixobactin resulted in accumulation of undecaprenyl-*N*-acetylmuramic acid pentapeptide (UDP-MurNAc-peptide), a precursor of the cell-wall peptidoglycan.[2] A similar observation was made in bacteria treated with vancomycin; however, while both teixobactin and vancomycin bind to lipid II, teixobactin's antibacterial activity against *E. faecalis* and *E. faecium* (VRE) suggests teixobactin binding to lipid II does not involve the peptide stem of lipid II, a distinguishing feature between these bacterial species.

Detailed *in vitro* studies have revealed that teixobactin operates through at least two distinct modes of action.[2] Teixobactin binds to precursors (lipid II, precursor for peptidoglycan biosynthesis, and lipid III, precursor for wall teichoic acid biosynthesis) (Figure 1C,D) of multiple cell wall biosynthetic pathways and inhibits the recycling of undecaprenyl pyrophosphate (C_{55}-PP), which is essential for lipid II and lipid III biosynthesis. Lipid II is a readily accessible, validated target for antibiotics such as vancomycin. Only a limited amount of lipid II can be synthesized at a time by the bacteria through the lipid II biosynthetic cycle[4] which is the key limitation in bacterial cell wall synthesis, making lipid II a very desirable target for antibiotic development. By binding to and trapping discrete, late-stage cell wall intermediates instead of protein targets, teixobactin curtails genetic alteration of its drug target in pathogenic bacteria, a common mechanism of antimicrobial resistance.[2]

The excellent bactericidal activity of teixobactin is reported to be due to not only inhibition of peptidoglycan synthesis but also the synergistic inhibition of wall teichoic acid synthesis.[3] The binding of teixobactin to lipid III, a key building block in the synthesis of wall teichoic acid, reduces the binding of autolysins (lytic enzymes) resulting in cleavage of intact peptidoglycan and cell death.[3]

Binding studies of teixobactin on purified cell wall precursors revealed that the antibiotic binds in 2:1 (teixobactin/lipid) molar ratio with lipid I, lipid II, lipid II (D-lac), lipid III, and

Received: July 20, 2017

DOI: 10.1021/acsinfecdis.7b00108
ACS Infect. Dis. XXXX, XXX, XXX−XXX

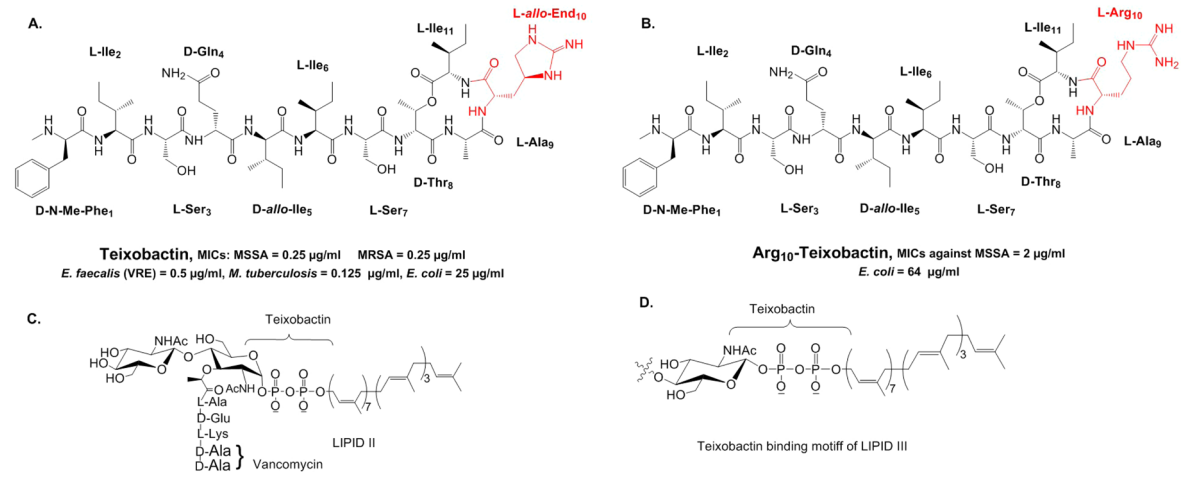

Figure 1. (A) Structure of teixobactin[2] and its analogue. (B) Arg_{10}-teixobactin.[6,7] Molecular targets of teixobactin, the bacterial cell wall precursor. (C) Lipid II[2] and wall teichoic acid precursor. (D) Lipid III.[2]

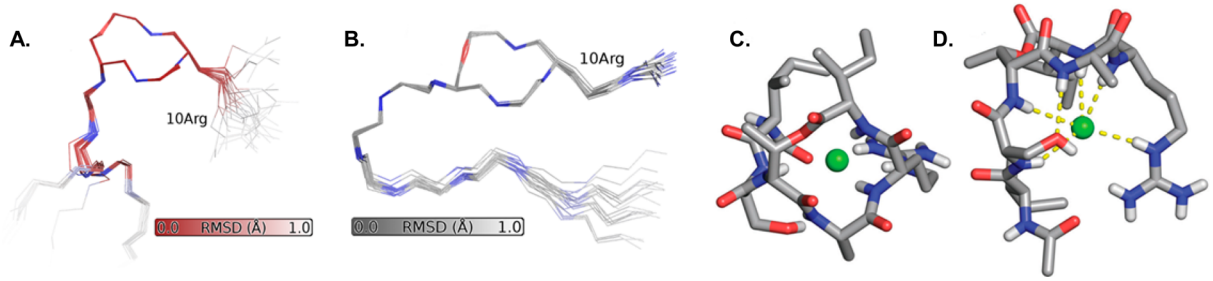

Figure 2. (A) Structure of Arg_{10}-teixobactin exhibiting native stereochemistry. (B) Structure of Arg_{10}-teixobactin comprised of only L-amino acids.[5] (C, D) X-ray crystallographic structure of Ac-Δ_{1-5}-Arg_{10}-teixobactin as the hydrochloride salt showing the top and side views, respectively.[11] Images reproduced with permission from refs 5 and 11. Copyright 2017 The Royal Society of Chemistry.

undecaprenyl pyrophosphate.[2] The pyrophosphate moiety and the first sugar moiety (either *N*-acetylmuramic acid or *N*-acetylglucosamine) were originally reported to play important roles in binding of teixobactin to most of these precursors. We previously reported the binding of teixobactin analogues (Figure 1B) to lipid II and the simplified geranyl pyrophosphate.[5] The minimal binding motif for teixobactin and its analogues is likely the pyrophosphate moiety and the polyprenyl chain, which is common in all these cell wall building blocks. This binding element is highly conserved in Gram-positive bacterial cell wall biosynthesis. The distinct mode of binding of teixobactin is responsible for its potent activity against vancomycin resistant bacteria, capable of solely mutating the peptidyl portion of lipid II.

Teixobactin showed good serum and microsomal stability and low cytotoxicity *in vitro*. Teixobactin is efficacious *in vivo*, displaying antibacterial activity in three mouse models of infection (the protective dose (PD_{50}) of teixobactin in mouse septicemia model was 0.2 mg/kg). While these results are encouraging, there remains a significant amount of work to be done to develop teixobactin as an antimicrobial therapeutic agent for human use. There is a pressing need for analogs of the original natural product to address common development issues such as potential off-target toxicity and metabolic liabilities in humans. Additionally, teixobactin's utility is limited by its low bioavailability, necessitating intravenous delivery. Access to analogs that may potentially resolve these issues is precluded by the lengthy and daunting synthesis of the teixobactin scaffold, highlighting the need for novel simplified molecules based on the natural product.

Teixobactin research has gained significant interest in the past year with several accounts being published describing the total synthesis of teixobactin[8,9] and its analogues.[6,7,10] Currently, there is no ideal synthetic route for teixobactin and its analogs, which contain the unusual amino acid L-*allo*-enduracididine. This amino acid is reported to be important for the potent antibacterial activity of teixobactin; however, it also represents a bottleneck in the development of teixobactin analogues due to various challenges pertaining to this residue's synthesis.[8] Several research groups, including our own, recently reported the synthesis of simplified teixobactin analogues in which the L-*allo*-enduracididine is replaced with structurally similar building blocks such as L-arginine and L-lysine. Encouragingly, these novel analogues possess a similar antimicrobial profile to teixobactin against *S. aureus* but can be accessed through a greatly simplified synthetic route from commercially available precursors.[6,7,10]

To develop highly potent derivatives of teixobactin, it was important to gain an understanding of the structure−activity relationship (SAR) of the nonribosomal peptide's interaction with its lipid targets. To this end, we reported the total syntheses and biological evaluation of the D and L analogues of Arg_{10}-teixobactin.[5] We successfully defined the three-dimensional molecular structure of seven teixobactin analogues (as determined via NMR) and reported that the disordered structure of teixobactin analogues is vital for their biological activity. The direct 3D structural comparison of two important

DOI: 10.1021/acsinfecdis.7b00108
ACS Infect. Dis. XXXX, XXX, XXX−XXX

derivatives, namely, Arg_{10}-teixobactin (Figure 2A) and Arg_{10}-teixobactin, with all L-amino acids (Figure 2B) revealed that only the former is disordered in its Arg_{10} and N-terminus and therefore active whereas the later forms a hairpin structure and folding onto itself, which presumably precludes cell wall intermediate binding. The amino acid configurations of the four D-amino acids (D-*N*-Me-Phe_1, D-Gln_4, D-*allo*-Ile_5, and D-Thr_8) are critical for biological activity, and inverting their configuration from D to L leads to a significant loss in antibacterial activity. Among the individual amino acids, D-Gln_4 is essential and D-*allo*-Ile_5 is important to maintain the disordered structure. Very recently, Nowick and co-workers reported the X-ray crystallographic structure of a truncated teixobactin analogue[11] (Figure 2C,D) in which the hydrophobic as well as the hydrogen bonding interactions present in the cavity structure containing a chloride ion were shown. Interestingly, the arginine residue of this truncated analogue provides a key interaction with the chloride ion via a folded conformation in the crystal structure (Figure 2D). Access to this conformation may only be granted through analogs with conformational flexibility, as shown in our NMR analysis (*vida supra*, Figure 2A). On the basis of the crystal structure, it has also been hypothesized that the core ring structure of teixobactin could encompass an anionic molecule such as a pyrophosphate, but stronger evidence of this has yet to be reported.

Nowick and co-workers[10] reported that any modification of configuration of the residues in the core ring structure of teixobactin results in a significant attenuation in biological activity. While pursuing the synthesis and development teixobactin analogues, the Albericio group performed a lysine scan of Arg_{10}-teixobactin.[12] They revealed that all four isoleucine residues in teixobactin are critical for biological activity. However, the L-Ser_3, D-Gln_4, and Ala_9 residues can be substituted with the corresponding enantiomer of lysine, and these analogues have comparable biological activity to that of Arg_{10}-teixobactin.

A thorough model for teixobactin activity and potential mechanisms of resistance is still in its infancy. An understanding of discrete lipid II interactions with teixobactin and its analogues at the molecular level is currently lacking, and more structural information will be crucial for the rational design of more potent analogues of teixobactin. The preliminary resistance studies of teixobactin against *S. aureus* and *M. tuberculosis* have indeed been very promising.[2] However, there is a need for rigorous resistance screening against teixobactin in different pathogens such as MRSA and VRE, which are already resistant to many antibiotics. One might argue that soil bacteria, which live close to teixobactin producer *E. terrae*, are most likely to have already acquired resistance to teixobactin and, thus, could potentially transfer their resistance by horizontal transmission. These mechanisms of resistance could arise through expression of teixobactin modifying enzymes or mutations that limit the access of teixobactin to lipid II by thickening the outer cell wall (such as those seen providing resistance against lantibiotics).

Although teixobactin biology and chemistry have made significant progress in the two years since the molecule's discovery, much remains unknown and unexplored. Questions still persist about the exact mechanism of cell wall intermediate sequestration, such as: Where in the microbial cell does this occur? And, why is a 2:1 ratio favoring teixobactin over lipid optimal? Additionally, several barriers to wide clinical use still exist, including: a lack of both oral bioavailability and activity against Gram-negative bacteria. Finally, the ability to predict resistance mechanisms before clinical testing would prove essential in the successful, controlled use of this powerful antibiotic. In this regard, exploring the strategies for teixobactin resistance in soil microbes and in clinically relevant bacterial strains (MRSA, VRE) would provide vital clues into potential resistance liabilities in the future. Through the careful analysis of teixobactin via new analogs, focused chemical–biology approaches, and rigorous resistance studies, there is hope that teixobactin can be translated to lessons and rewards for future antibiotic drug therapy.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Dr. Abhishek Iyer for his assistance in preparation of the manuscript and Dr. Graham Lappin and Professor Courtney C. Aldrich for their comments on the manuscript.

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DOI: 10.1021/acsinfecdis.7b00108
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